**Table VII.** 2-Halo-4-methoxyphenylalanines

| Compd | X | Mp, °C dec | Yield, % | Formula | Analyses |
|---|---|---|---|---|---|
| 10 | Cl | 245–247 | 81 | $C_{10}H_{12}ClNO_3 \cdot HCl$ | C, H, N |
| 11 | Br | 249–250 | 88 | $C_{10}H_{12}BrNO_3 \cdot HCl$ | C, H, N |
| 12 | F | 216–226[a] | 56 | $C_{10}H_{12}FNO_3$ | C, H, N |

[a] Reported[6] mp 217–221° dec.

VII, 10–12). Compound 4 (1.0 g, 0.0027 mol) was hydrolyzed with 20 ml of 6 *N* HCl at reflux for 6 hr. After the reaction mixture cooled to 25°, the salt which separated was collected by filtration. Recrystallization of the HCl salts of **10** and **11** gave analytical samples (Table VII); however, the salt of **12**, mp 186–189° dec, was converted to the free base by neutralization with concentrated $NH_4OH$.

**3-Chloro-L-tyrosine (13).** This compound was obtained from K & K Laboratories, Plainview, N.Y., and was recrystallized from aqueous ethanol and dried prior to its use in the microbiological assays.

**3-Bromo-DL-tyrosine (14).** This compound was prepared by adding an equivalent amount of a bromination solution (154 mg of $KBrO_3$ and 548 mg of KBr in 100 ml of $H_2O$) to a solution of 500 mg of DL-tyrosine in 0.5 *N* HCl. The solution was concentrated *in vacuo* to a few milliliters and let stand at 25°. The precipitated hydrohalide salts were collected by filtration and dried: yield 260 mg. In a separate experiment, the pH of an aqueous solution of 109 mg of hydrohalide salts was adjusted to 7 with 10% aqueous NaOH to yield 76 mg of the free base of 3-bromotyrosine, mp 245–246° (lit.[10] mp 247–248° *via* a different procedure).

**3-Fluoro-DL-tyrosine (15).** This compound was purchased from the Aldrich Chemical Co., Inc., Milwaukee, Wis., and was of sufficient purity to be used as the reference compound in the microbiological assays.

**Microbiological Assays.** For the growth inhibition and tyrosine reversal studies with *E. coli* (ATCC 9723) a previously described assay procedure[11] and inorganic salts–glucose medium[12] were used. For the growth assays with *S. faecalis* (ATCC 8043) and *L. plantarum* (ATCC 8014, *L. arabinosus* 17-5), the procedure and basal medium were the same as previously reported[13] except 100 μg/ml of calcium pantothenate was included in the vitamin supplement and both tyrosine and phenylalanine were omitted from the amino acid medium unless otherwise noted in the tables. The basal medium for *S. faecalis* was further modified by adding 20.0 μg/ml of glutamine under aseptic conditions.

The tyrosine analogs (10 mg) were dissolved in sterile $H_2O$ (10 ml) at 25°. From these solutions, serial dilutions were made to the desired concentrations and added aseptically to the previously autoclaved assay tubes without being heated. After inoculation, the assay tubes with *E. coli* were incubated at 37° for 16 hr, and those with *S. faecalis* and *L. plantarum* were incubated at 30° for 24 and 30 hr, respectively.

In all assays the amount of growth was determined spectrophotometrically at 600 mμ with a Bausch & Lomb Spectronic 20 in terms of absorbance readings of the turbid culture medium against a blank of uninoculated medium set at zero absorbance. The milligrams of dry weight of cells were calculated from a standard curve of milligrams of cells plotted *vs.* absorbance readings. In all the assays, appropriate controls were run and the results of the minimum inhibitory concentrations of the various compounds tested were shown to be reproducible on repeating the assays at least 12 times.

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# The Charge-Transfer Constant. A New Substituent Constant for Structure–Activity Relationships

Bogumil Hetnarski* and R. D. O'Brien

*Section of Neurobiology and Behavior, Cornell University, Ithaca, New York 14853.* *Received August 19, 1974*

A new, versatile constant accounting for the π-complex formation ability of aromatic systems is proposed. The constant is derived from charge-transfer complex data of aromatic species (charge-transfer constant, $C_T$). The applicability of $C_T$ in structure–reactivity relations is demonstrated. It is shown that the affinity of various inhibitors (32) and acetylcholinesterase is a function of $C_T$ and π (Hansch hydrophobicity constant).

The analysis of the relation between structure and activity for a variety of drugs and toxicants has been much improved by two advances. One is the development of appropriate substituent constants which measure the effect of a substituent upon such factors as electronic character, size, or hydrophobicity. The second is the development by Hansch[1] of the technique of multiple regression analysis, by which it is possible to evaluate a situation which has, as one might have expected, turned out to be the most frequent case, *i.e.*, that the effectiveness of an agent is influenced by a number of factors, each of which is differently affected by a change in substitution.

Recently,[2,3] in studies on the relationship between structure and activity of carbamates as inhibitors of acetylcholinesterase, we have provided evidence that the formation of a charge-transfer complex (CTC) with the enzyme may be involved; at the moment the evidence is only indirect, because substantial quantities of highly pu-

rified acetylcholinesterase are not yet available and because of the difficulty which may account for the failure to show such complexes with any protein; the new absorption typically involves only a simple residue and is not readily detected in the presence of background absorptions. Herein we describe a way to introduce a constant derived from CTC formation constants as one of the variables in multiple regression analysis. Our application is to cholinesterase inhibitors, but the principle should be generally applicable.

We shall deal only with the case of aromatic compounds and particularly those in which it is the $\pi$ electrons of the ring which are involved in donation to the complex. It should be noted that in some substituted phenyl compounds (such as aminophenyl derivatives) the donated electrons may derive from sources other than the ring, and these will constitute special cases not amenable to our treatment.

**Methods.** $K_d$ values were determined by the zero-time method.[4,5] The assays were performed at 25° by the following procedure, modified from Ellman, *et al.*[6] A mixture was prepared consisting of 15 vol of buffer (0.2 *M* sodium phosphate, pH 7.6), 0.2 vol of acetylcholine chloride (Sigma) in freshly distilled water, and 1 vol of a 0.01 *M* solution of 5,5′-dithiobis(2-nitrobenzoic acid) plus 0.01 *M* sodium bicarbonate in 0.2 *M* sodium phosphate buffer, pH 7.

For the majority of compounds the concentration of acetylcholine chloride was about $1 \times K_m$ ($K_m = 0.29 \times 10^{-3}$ *M*, found under analogous conditions). Of that mixture, 1.62 ml was placed into the cuvette and 1 ml of 1.5 units/ml of bovine erythrocyte AChE (Sterwin Chemicals) solution in the aforementioned buffer solution (pH 7.6) was added. The reaction was followed over 0.5 min at 412 nm using a Beckman Acta III spectrophotometer; then 0.02 ml of a methanol solution of inhibitor was added, the contents of the cuvette were mixed instantly, and readings were taken at 412 nm. In order to find $K_d$ values we plotted $i$ (inhibitor final concentration) against $(v_c/v_0) - 1$, where $v_c$ and $v_0$ are the reaction velocities in the absence and presence of an inhibitor, respectively. The $v_0$ data were obtained by extrapolating velocities of the substrate hydrolysis to zero time, using the plot of the logarithm of hydrolysis rate as a function of time.[7] The inhibitor concentrations ranged between $10^{-3}$ and $10^{-5}$ *M*.

$K_x$ values (association constants of CTC between aryl methylcarbamates and TCNE) were determined by the Benesi-Hildebrand method[8] in 1,2-dichloroethane at 23°.[9,10] The method involved the preparation of a series of concentrations of carbamates (acting as donors) in solvent, with mole fractions of from 0.003 to 0.007. Each was made $5 \times 10^{-3}$ *M* with respect to TCNE. The resultant absorption was measured and obeyed the Benesi-Hildebrand equation (for a 1-cm path length)

$$M_A/A = 1/\epsilon_c K_x C_D + 1/\epsilon_c \quad (1)$$

where $A$ is the observed absorption and $M_A$ is the molar concentration of acceptor, $\epsilon_c$ is the molar extinction coefficient of the complex, $K_x$ is the association constant of donor and acceptor, and $C_D$ is the concentration of donor (carbamate) in mole fractions. A plot of $M_A/A$ as a function of $1/C_D$ permitted the calculation of $K_x$, for which the least-squares method was used.

## Results and Discussion

Although many of the compounds we shall discuss are carbamylating agents, we shall discuss for all compounds only the first step of the overall reaction, *i.e.*, the binding to the enzyme surface, as measured by the dissociation constant $K_d$. We hypothesize that the variations in $K_d$ may be attributable to a small number of factors, each of which makes a free energy contribution, and that those contributions are linearly related, *i.e.*, they can be summed.

We have found that, in four series of inhibitors, the variations in affinity for the enzyme can be largely accounted for in terms of two factors only: variations in hydrophobicity and ability to donate $\pi$ electrons to form CTC's, as measured with a model acceptor (tetracyanoethylene) (Table I). The relative contribution of these two factors varies in the four series. We measure the hydrophobicity of each substituent by Hansch's constant $\pi$ defined[1,11] in a fashion analogous to the Hammet $\sigma$ constant

$$\pi_x = \log P_x - \log P_H \quad (2)$$

where $P_x$ is the partition coefficient (usually in the octanol-water system) of the substituted and $P_H$ of the unsubstituted compound.

We now define the charge-transfer constant

$$C_T = \log K_x - \log K_H \quad (3)$$

where $K_x$ is the association constant for CTC formation with tetracyanoethylene of the substituted and $K_H$ is that for the unsubstituted compound. Clearly other model acceptors could be used, and the principle is applied as readily to acceptor as to donor compounds. It may be the $C_T$, rather than being treated as analogous to $\pi$, should be considered, in a way analogous to the Hammett constant, as the product of two factors $\rho_c$ and $\sigma_c$, where $\rho_c$ is a reaction constant which can differ with different acceptors, and with different classes of donors, and where $\sigma_c$ is a true constant for the substituted aromatic residue. In practice we find, with the compounds discussed in this paper, that either (3) is correct or else $\rho$ is very similar for our four series. For instance, $C_T$ for a series of seven aromatic hydrocarbons were closely correlated ($r = 0.99$) with $C_T$ for the corresponding para-substituted phenylmethyl carbamates; what is more, the individual $C_T$ values were very close; for the unsubstituted compounds, the $C_T$ values were set at zero; for methyl, the values for the hydrocarbon and carbamates were 0.27 and 0.21; for chloro, −0.41 and −0.27; for iodo, −0.21 and −0.15; for naphthyl, 0.77 and 0.73. For present purposes, it is not necessary to establish whether a $\rho$ factor is involved, because structure-activity correlations are made within a given series, in which $\rho$ would be constant.

For a carbamate CX, where C is the methylcarbamyl group and X the leaving group, the overall reaction with enzyme (E) has three steps[4]

$$CX + E \xrightarrow{k_1} CXE \xrightarrow{k_2} CE + X \xrightarrow{k_3} C + X + E \quad (4)$$

The affinity for the enzyme bovine erythrocyte acetylcholinesterase was measured as $K_d$, the dissociation constant, by the method of Hart and O'Brien.[5] It should be noted that in the carbamylating carbamates (which constitute two of the classes described herein) the $K_d$ controls only one step of the overall inhibitory reaction, *i.e.*, the formation of complex between inhibitor and enzyme, and subsequent steps of carbamylation and decarbamylation are involved in the total and recovery reaction.

There are two approaches to determining $C_T$. Firstly, one can measure for every compound the association constant ($K_x$) with tetracyanoethylene and compare it with the unsubstituted parent. This was done by us for compounds **1-20**, using the Benesi-Hildebrand method[8-10]

**Table I**

| No. | R | $K_d$ emp, m$M$ | $K_d$ calcd, m$M$ | $K_x$ (mole fraction)$^{-1}$ | $\lambda_{max}$, nm | $C_T$ | $\pi^a$ |
|---|---|---|---|---|---|---|---|
| | | Aryl Methylcarbamates ROC(O)NHCH$_3$ | | | | | |
| 1 | 4-Fluorophenyl | 6.49 ± 1.30 | 6.91 | 5.5 ± 2.1 | 357 | −0.41 | 0.15 |
| 2 | 4-Chlorophenyl | 4.51 ± 0.96 | 4.97 | 7.6 ± 1.5 | 360 | −0.27 | 0.70 |
| 3 | 4-Bromophenyl | 3.80 ± 0.16 | 3.33 | 12.9 ± 1.0 | 400 | −0.04 | 1.02 |
| 4 | 4-Iodophenyl | 3.32 ± 0.23 | 3.07 | 10.0 ± 6.2 | 420 | −0.15 | 1.26 |
| 5 | Phenyl | 6.05 ± 0.85 | 5.88 | 14.2 ± 3.6 | 365 | 0.00 | 0.00 |
| 6 | 4-Tolyl | 3.92 ± 0.36 | 3.78 | 23.1 ± 3.9 | 405 | 0.21 | 0.52 |
| 7 | 4-Anisyl | 4.71 ± 0.26 | 4.56 | 36.4 ± 3.1 | 510 | 0.41 | −0.04 |
| 8 | 4-Ethylphenyl | 3.18 ± 0.07 | 2.80 | 19.9 ± 3.5 | 405 | 0.15 | 0.97 |
| 9 | 4-Isopropylphenyl | 0.85 ± 0.22 | 1.00 | 31.1 ± 1.7 | 405 | 0.34 | 1.40 |
| 10 | 4-Butoxyphenyl | 0.34 ± 0.01 | 0.49 | 39.1 ± 3.0 | 525 | 0.44 | 1.46 |
| 11 | 1-Naphthyl | 0.013 ± 0.001 | 0.38 | 75.5 ± 10.6 | 540 | 0.73 | 1.12 |
| 12 | 3-Fluorophenyl | 2.51 ± 0.16 | 2.70 | 5.6 ± 1.4 | 360 | −0.42 | 0.13 |
| 13 | 3-Chlorophenyl | 1.87 ± 0.12 | 1.61 | 4.2 ± 1.9 | 365 | −0.53 | 0.76 |
| 14 | 3-Bromophenyl | 0.77 ± 0.08 | 0.60 | 14.2 ± 1.1 | 390 | 0.00 | 0.94 |
| 15 | 3-Iodophenyl | 0.11 ± 0.01 | 0.36 | 10.3 ± 1.6 | 395 | −0.14 | 1.15 |
| 16 | 3-Tolyl | 1.49 ± 0.49 | 1.09 | 27.7 ± 3.7 | 400 | 0.29 | 0.51 |
| 17 | 3-Ethylphenyl | 0.034 ± 0.06 | 0.42 | 17.7 ± 2.6 | 400 | 0.10 | 0.97 |
| 18 | 3-Isopropylphenyl | 0.0028 ± 0.0003 | −0.25 | 18.5 ± 6.4 | 400 | 0.12 | 1.30 |
| 19 | 3-Anisyl | 1.74 ± 0.03 | 1.78 | 31.9 ± 8.2 | 495 | 0.35 | 0.12 |
| 20 | 3-Isopropoxyphenyl | 0.020 ± 0.0001 | 0.24 | 30.2 ± 2.3 | 490 | 0.33 | 0.92 |
| | | Arylmethyl Methylcarbamates RCH$_2$OC(O)NHCH$_3$ | | | | | |
| 21 | 4-Chlorophenyl-methyl | 3.10[b] | 3.19 | 12.0[c] | 340 | −0.41 | 0.71 |
| 22 | Phenylmethyl | 2.80 | 2.80 | 31.1 | 350 | 0.00 | 0.00 |
| 23 | 4-Tolylmethyl | 2.35 | 1.83 | 57.6 | 395 | 0.27 | 0.56 |
| 24 | 4-Anisylmethyl | 2.30 | 2.26 | 68.7 | 520 | 0.35 | −0.02 |
| 25 | 1-Naphthylmethyl | 0.13 | 0.39 | 182.0 | 545 | 0.77 | 0.88 |
| | | Aromatic Hydrocarbons RH | | | | | |
| 26 | Chlorobenzene | 3.80 ± 0.47 | 3.85 | 12.0[d] | 379[e] | −0.41 | 0.71 |
| 27 | Benzene | 3.93 ± 0.77 | 3.75 | 31.1 | 384 | 0.00 | 0.00 |
| 28 | Toluene | 2.30 ± 0.30 | 2.26 | 57.6 | 406 | 0.27 | 0.56 |
| 29 | Anisole | 2.69 ± 0.34 | 2.86 | 68.7 | 507 | 0.35 | −0.02 |
| 30 | *p*-Xylene | 0.68 ± 0.01 | 0.79 | 119.0 | 460 | 0.59 | 1.02 |
| 31 | *m*-Xylene | 1.04 ± 0.25 | 1.01 | 93.2 | 440 | 0.48 | 1.07 |
| 32 | Naphthalene | 0.60 ± 0.08 | 0.51 | 182.0 | 550 | 0.77 | 0.88 |

[a] $\pi$ values are calculated on the basis of reported literature partition coefficients in the system octanol-water.[11,19] [b] Data reported by Hetnarski and O'Brien.[3] [c] As $K_x$ for arylmethyl methylcarbamates were employed association constants of their aromatic portions with TCNE as reported by Briegleb.[13] [d,e] Data given by Briegleb.[13]

with 1,2-dichloroethane as solvent at 23°. The method involves measurement of the intensity of charge-transfer absorption bands at different component concentrations. For the hydrocarbons, compounds **26–32**, the values were taken from Briegleb.[13] Secondly, if the value of $C_T$ is truly a constant in different series, then one should be able to use the $C_T$ values from any one series, *e.g.*, the hydrocarbon parents, for any other series. And indeed we have reported[3] that for the arylmethyl methylcarbamates (compounds **21–25**) the use of hydrocarbon values provides an excellent correlation between $K_x$ and $K_d$, the dissociation constant between enzyme and inhibitor. Consequently, Table I uses the values for the hydrocarbon parent for these five compounds. A test of the validity of this approach is that the $C_T$ values obtained by actual measurements (in the series **1–20**) agree well with the available $C_T$ values calculated from literature values on the hydrocarbon parents (compounds **26–32**), even though our measurements were at 23° in dichloroethane and the literature values were from experiments at 22° in dichloromethane. The values for compound **2** and its hydrocarbon parent **26** were −0.27 and −0.41; for **6** and **28**, 0.21 and 0.27; for **7** and **29**, 0.41 and 0.35; for **11** and **32**, 0.73 and 0.77.

One approach to the formulation of the problem is to propose that each increment in the bonding energy of the substituted as compared with the unsubstituted compound is due to the sum of the changes in free energy related to CTC formation and to hydrophobic binding. The increment in bonding energy can conveniently be measured by $C_A$, defined as

$$C_A = \log K_{a(x)} - \log K_{a(H)} \quad (5)$$

where $K_{a(x)}$ is the affinity ($1/K_d$) of the substituted compound in any one of the four series to be described, and $K_{a(H)}$ is that of the unsubstituted parent compound. The simplest anticipation would be that

$$C_A = k_1 C_T + k_2 \pi + k_3 \quad (6)$$

We explored the following: series A, para-substituted phenyl methylcarbamates plus carbaryl (*i.e.*, 1-naphthyl methylcarbamate); B, meta-substituted phenyl methylcarbamates; C, para-substituted arylmethyl methylcarba-

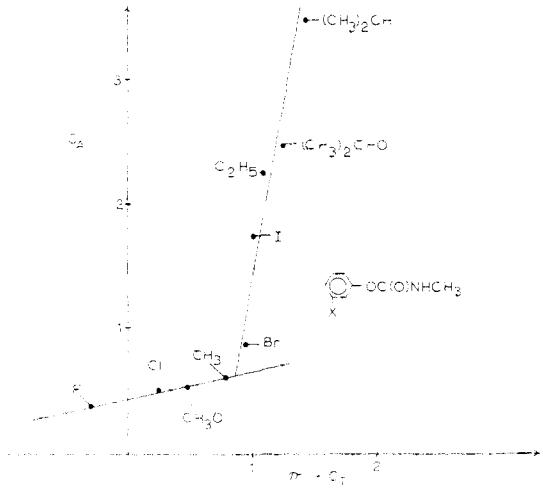


**Figure 1.** Plot of $C_A = f(C_T + \pi)$.

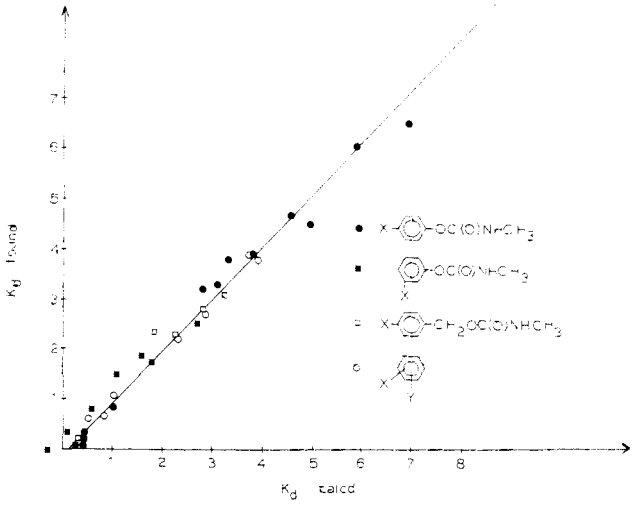


**Figure 2.** Diagram of $K_d$ found *vs.* $K_d$ calculated.

mates; D, substituted aromatic hydrocarbons. For each series, plotting $C_A$ as a function of $\pi$ and $C_T$ on a three-dimensional device revealed two separate planar components. For example, in series B, for the first four compounds (F, Cl, $CH_3O$, and $CH_3$ derivatives) the correlation coefficient $r$ for eq 5 was 0.999 and for the second five [Br, I, $C_2H_5$, $(CH_3)_2CHO$, and $(CH_3)_2CH$] it was 0.956. These good correlations contrast with $r$ = 0.831 for the whole series. Because the $\pi$ and $C_T$ are independent vectors which vary in the same direction, it was possible to observe (Figure 1) the broken-line relation on a two-dimensional plot of $C_A$ as a function of $(C_T + \pi)$.

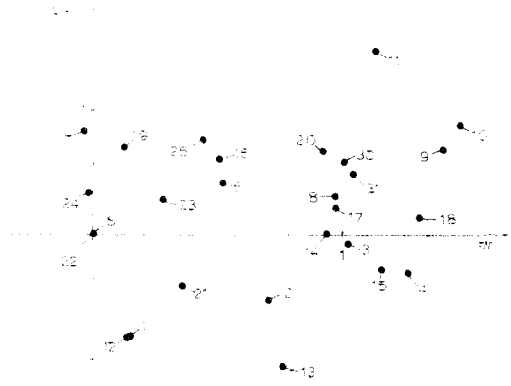

**Figure 3.** Diagram of $C_T$ vs. $\pi$ (lack of correlation).

The broken-line three-dimensional plots are well linearized by semilogarithmic plotting. With a computerized least-squares method we found for series A–D the following relations

$$K_d = -(3.476 \pm 0.359)C_T - (2.645 \pm 0.221)\pi + 5.88 \quad (7)$$
$$r = 0.989;\ n = 11;\ s = 0.367$$

$$K_d = -(1.228 \pm 0.357)C_T - (1.960 \pm 0.271)\pi + 2.444 \quad (8)$$
$$r = 0.958;\ n = 9;\ s = 0.322$$

$$K_d = -(2.230 \pm 0.510)C_T - (1.038 \pm 0.540)\pi + 3.016 \quad (9)$$
$$r = 0.964;\ n = 5;\ s = 0.442$$

$$K_d = -(2.632 \pm 0.149)C_T - (1.385 \pm 0.130)\pi + 3.751 \quad (10)$$
$$r = 0.997;\ n = 7;\ s = 0.134$$

where $n$ is the number of compounds and $s$ the standard deviation. From these equations, we were able to calculate a value of $K_d$ for all 32 compounds and compare them with the measured values. Figure 2 shows that there was an excellent correlation ($r$ = 0.975).

In order to determine which of the two variables ($\pi$, $C_T$) is more significant, we found equations for $K_d = f(C_T)$ and $K_d = f(\pi)$ for each of the four groups of inhibitors which were investigated and calculated relevant variances and $F$ values (Table II). The $F$ values for all four groups of inhibitors show that the relative differences in their variances are insignificant. $C_T$ and $\pi$ are almost equally important for $K_d$ predictions. While there exists no correlation between $C_T$ and $\pi$ (Figure 3), some dependency oc-

Table II

| Inhibitors | $K_d(C_T)$ variance $[K_d = f(C_T)]$ | $K_d(\pi)$ variance $[K_d = f(\pi)]$ | Deg of freedom (identical for numerator and denominator) | $F$ | $F$ values for 95% probability of difference between the variances |
|---|---|---|---|---|---|
| Para-substituted phenyl methylcarbamates | 2.01 | 1.40 | 10 | 1.43 | 2.97 |
| Meta-substituted phenyl methylcarbamates | 0.75 | 0.23 | 8 | 3.26 | 3.44 |
| Benzyl methylcarbamates | 0.28 | 1.03 | 4 | 3.68 | 6.39 |
| Aromatic hydrocarbons | 0.36 | 0.91 | 6 | 2.53 | 4.28 |

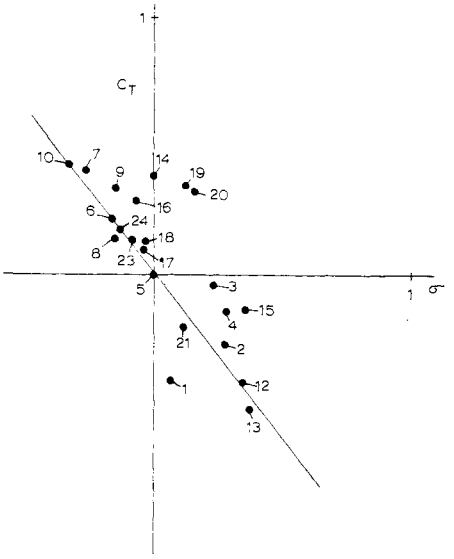


**Figure 4.** Diagram of $C_T$ *vs.* $\sigma$.

curs between $C_T$ and $\sigma$ (Figure 4). The following correlation matrix shows the collinearity among $\pi$, $C_T$, and $\sigma$.

| | $C_T$ | $\pi$ | $\sigma$ |
|---|---|---|---|
| $C_T$ | $r(C_TC_T) = 1$ | $r(C_T\pi) = 0.191$ | $r(C_T\sigma) = -0.755$ |
| $\pi$ | $r(\pi C_T) = 0.191$ | $r(\pi\pi) = 1$ | $r(\pi\sigma) = 0.119$ |
| $\sigma$ | $r(\sigma C_T) = -0.755$ | $r(\sigma\pi) = 0.119$ | $r(\sigma\sigma) = 1$ |

There is a parallel between the values of $C_T$ and of Hammett's $\sigma$ in many cases: substituents which withdraw electrons from the $\pi$-electron system (whose availability is what $C_T$ primarily measures) will also modify the reactivity of a given reaction center (which is what $\sigma$ measures). However, $C_T$ also reflects a suitable steric situation dependent on donor–acceptor fit; it is applicable to ortho-, meta-, and para-substituted phenyl compounds; can be applied to other aromatic systems than benzene (because of limited application of $\sigma$, we could not include naphthalene in Figure 4) such as the three-dimensional systems, *e.g.*, metallocenes; is a direct measure of the capacity of a given compound to act as a donor in $\pi$-electron complexes; and is particularly easy to measure when the complexes absorb in the ultraviolet or visible regions. It should be noted that if a substituent is a potential electron donor itself (*e.g.*, $-NH_2$, $-SCH_3$) one cannot be assured that the bonding electrons derive from the aromatic ring.[14] In addition, CTC's will not be formed with rings which are polysubstituted with high electronegative substituents, such as $NO_2$ or CN, and are therefore better electron acceptors than the typical model acceptors, such as tetracyanoethylene and tetracyanoquinodimethane.

The reported ability for simple aromatic hydrocarbons to inhibit $\alpha$-chymotrypsin[15] and other enzymes[16] might well be due to CTC formation but one should not expect simple correlations between CTC forming ability and potency, as has previously been sought for such activities as carcinogenesis[17] or sedative activity.[18] The usual case will be that formation of CTC is one factor of importance but must be coupled with distributional and reactivity factors if a full accounting of the variability of potency is to be given.

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